Getting to the heart of the matter: role of *Streptococcus mutans* adhesin Cnm in systemic disease

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Keywords: *Streptococcus*; infective endocarditis; adhesin; collagen-binding protein; *Lactococcus lactis*

Streptococcus mutans is one of an estimated 700 prokaryote species that are recognised as constituents of the oral microbiota [1]. *S. mutans* is exclusively found as a member of the polymicrobial biofilm communities that comprise dental plaque [2], and is perhaps most notorious as the first bacterium to be identified as a major aetiological agent of dental caries (tooth decay) [3]. The incidence of this disease has declined with the introduction of population-based prevention measures [4]. Nonetheless, dental caries remains one of the most ubiquitous bacterial infections of humans and represents a significant financial burden to the healthcare system [5, 6].

S. mutans and several other streptococcal species that commonly inhabit the oral cavity are collectively known as the 'viridans group' streptococci and together can comprise up to 80% of early dental plaque [7, 8]. Outside of the oral niche, however, this group of bacteria is also particularly recognised for its association with heart condition infective endocarditis (IE). Together with staphylococci and enterococci, the viridans group streptococci account for 80-90% of all IE cases [9]. Whilst relatively rare (3-10 cases per 100,000 individuals), the one year mortality rate for this infection of the heart valves remains at ca. 30%, with treatment options frequently incorporating surgery and long-term administration of antibiotics [10]. Given the current crisis of increasing incidence in antibiotic resistance within the global microbial population [11], there is considerable pressure to devise alternative treatment strategies for IE. To achieve this, however, greater understanding of the pathogenic mechanisms that underpin this disease is required.

For the viridans group streptococci, the initial step in IE pathogenesis is bacterial entry into the bloodstream. Such transient bacteraemias arise following disruption of the oral mucosae, often simply as a result of daily practices (e.g. toothbrushing, flossing) [12-15]. Upon transiting from the oral cavity to the cardiovascular system, these streptococci must then adhere to the endothelium of the heart valves, where they promote deposition of fibrin and blood platelets to form an infective vegetation (clot) [9, 16, 17]. In this issue, Freires et al. [18] utilise a surrogate host expression system to demonstrate that *S. mutans* surface adhesin Cnm (collagen-binding protein of *S. mutans*) is essential for this process.

Strains of *S. mutans* can be divided across four capsular polysaccharide serotypes (*c*, *e*, *f* and *k*), of which serotype *c* is the most prevalent within the oral niche [19, 20]. Serotypes *e*, *f* and *k* have been found to express Cnm or the closely-related collagen-binding protein Cbm. By contrast, serotype *c* strains, which comprise ca. 75% of isolates, typically lack the *cnm* locus [21-24]. Intriguingly, the less abundant serotypes are highly overrepresented among isolates associated with *S. mutans* extra-oral infections [25, 26]. This disparity in distribution provided some of the first evidence that such collagen-binding adhesins may make a critical contribution to the capacity for *S. mutans* to cause systemic disease. Cnm has been shown to promote attachment to extracellular matrix (ECM) proteins collagen (types I, II, III and IV) and laminin [21, 25, 27-29], and to facilitate invasion of cardiac endothelial cells [28, 30]. The role of Cnm as a potential virulence factor was also demonstrated using the *Galleria mellonella* wax worm model of systemic infection [28, 30]. Such studies were primarily performed using *S. mutans* Δ*cnm* knockout mutants and corresponding complemented strains. However, *Streptococcus* bacteria are notorious for exhibiting adhesin redundancy [31-33]. This feature likely contributes to the overwhelming success of these bacteria as host colonisers, but can make it challenging to unequivocally ascribe an adhesive function(s) to a specific protein. One way to address this issue is to utilise a heterologous expression system and a successful strain for which

there is precedent with *Streptococcus* proteins is *Lactococcus lactis* [34-38]. As a Gram-positive coccus, *L. lactis* shares many of the systems required for surface protein export and display and yet, as a dairy industry starter microorganism, it lacks capacity to interact strongly with human cells and tissues [39]. Consequently, *L. lactis* can serve as an excellent 'blank canvas' with which gain of function can be explored following expression of a heterologous protein.

Using *L. lactis* expressing Cnm, Freires et al. [18] demonstrate unambiguously that this adhesin mediates adhesion to ECM components collagen type I and laminin, by both direct whole cell binding assays and complementary inhibition studies using anti-Cnm serum. Cnm is also shown to confer capacity to invade human coronary artery endothelial cells (HCAEC), and Cnm^+ *L. lactis* exhibits significantly enhanced virulence using the *G. mellonella* model of systemic disease compared to parent strain. Additionally, in contrast to parent strain, *L. lactis* expressing Cnm is able to bind freshly extirpated human aortic valve tissue. Evidence suggests that one way IE might be initiated is through adherence of viridans group streptococci to exposed ECM proteins of damaged heart valves [40]. The SEM images shown in Freires et al. [18] support this mechanism, with bacterial adhesion to collagenous fibrils present in areas of damage clearly visible. Nonetheless, this study also presents examples of binding to supposedly intact endothelium. Such observations are of interest, as they imply that Cnm may also facilitate recognition of endothelial receptors. Direct binding to endothelial cell lines in vitro has been demonstrated previously for *Streptococcus* bacteria [41-44], but this has yet to be considered as a potential mechanism in IE. Such a possibility is worthy of investigation in future studies.

Evidence of a role for specific streptococcal adhesins in IE has proven difficult to obtain using animal models, possibly reflecting the challenge posed by adhesin redundancy and/or the capacity for bacteria to utilise multiple mechanisms. The most striking example of this perhaps was seen for viridans group member *Streptococcus sanguinis*, for which no single deletion of any of its 33 surface

(LPxTG-anchored) proteins significantly affected IE outcome [45]. Again, this is where a surrogate host can offer advantages. Using a rabbit model of IE in which the animals are co-inoculated with both parent and Cnm$^+$ *L. lactis*, Freires et al. [18] show that Cnm confers a 67% increase in infectivity. This serves to reinforce the proposed role of Cnm as enabling initial contact and retention of *S. mutans* with valve endocardium.

While surface expression of heterologous proteins can be successfully achieved with *L. lactis*, one aspect that may not be faithfully reproduced is with posttranslational modifications such as glycosylation. This can, however, in itself be informative. In *S. mutans* Cnm has been shown to be co-transcribed with GT-A type glycosyltransferase PgfS, which appears to modify Cnm through *O*-glycosylation of its threonine-rich B domain [46]. Freires et al. [18] show that this glycosylation does not occur in *L. lactis*, resulting in expression of a lower MW variant of Cnm with greater susceptibility to proteinase K degradation. Since Cnm$^+$ *L. lactis* exhibits significant interactions with ECM proteins and cardiac endothelium, these data indicate that it is the protein backbone rather than the sugar modifications of Cnm that mediates its adhesive properties. Nonetheless, this study also provides evidence that the stability conferred by *O*-glycosylation may be critical for Cnm-mediated adhesion in vivo. Deciphering the precise contribution that *O*-glycosylation makes to the overall functionality of Cnm in adhesion and pathogenesis again represent important areas for future research.

It is becoming increasingly evident that a diverse array of mechanisms are utilised by different members of the viridans group streptococci to promote thrombosis and the progression of IE. Studies such as these of Freires et al. [18] are helping to advance understanding of this complex host-microbe interplay and the development of new anti-infection strategies that might help move away from the current complete reliance on antibiotics.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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